Human Aqueous Humor Phosphatase Activity in Cataract and Glaucoma

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PURPOSE. To investigate the presence and activity of protein phosphatase-2A (PPase2A), protein phosphatase-2C (PPase2C), and protein tyrosine phosphatases (PTPs) in the human aqueous humor (AH) of patients with primary open-angle glaucoma (POAG) and cataract and to study the correlation between these phosphatases and the redox state of the AH.

METHODS. Eighty-six cataract patients and 29 POAG patients who were scheduled for cataract surgery with or without glaucoma surgery were enrolled in the study. PPase2A, PPase2C, and PTP levels in AH were measured by enzymelinked immunosorbent assays, Western blot analyses, and spectral methods. The redox state was measured by spectral and fluorescent methods.

RESULTS. Phosphatase activity–positive results were significantly higher in AH samples from the POAG group (PP2A $\chi^2(1) = 11.754, P < 0.01$; PP2C $\chi^2(1) = 8.754, P < 0.01$; PTP $\chi^2(1) = 11.073, P < 0.01$). Western blot analysis revealed higher PP2C levels in the AH of glaucoma patients compared with PP2C levels in the AH of cataract patients ($P = 0.012$). Both oxidized/reduced glutathione ratios and superoxide dismutase levels in the AH were significantly higher in the glaucoma group than in the cataract group. Finally significant correlations were found between PP2A and PP2C, PP2A and PTP, and total antioxidant activity and PTP levels.

CONCLUSIONS. There is a statistically significant difference between phosphatase levels in the AH of POAG patients and cataract patients. The phosphatase content of the AH represents tissue pathology, but their presence in the AH may be attributed to cell debris or to active signaling to other molecular events. (Invest Ophthalmol Vis Sci. 2012;53:1679–1684) DOI:10.1167/iovs.11-9120

The secretion of aqueous humor (AH) and the regulation of its outflow are physiologically important processes for normal eye function and for maintaining intraocular pressure (IOP) within the normal range. Complex mechanisms regulate AH circulation; impairment in AH outflow results in elevation of IOP and leads to the development of glaucoma. The protein composition and antioxidant status of the AH change dramatically in different ocular maladies such as myopia,2–3 uveitis,2–3 cataract,4–5 and glaucoma.6–8 AH antioxidant capacity reflects the degree of oxidative stress in the surrounding tissues.9 The antioxidant capacity of AH in congenital glaucomatous rabbits was shown to be significantly lower than that of control rabbits.10 Furthermore, a significant change in small molecular weight antioxidant content in glaucoma was accompanied by a decrease in antioxidant enzyme activity.11 Beyond the direct damage associated with the exposure of tissues and cells to oxidative stress, several signaling pathways that lead to cell rescue or apoptosis, gene expression, and protein activity changes, may be triggered. For example, the ability of oxidative stress to stimulate the immune system is well established and has been shown to take place in the retina and optic nerve head glia.12

The redox state of tissues and cells affects the delicate balance between phosphatases and kinases3 that is made up of intricate networks of signaling pathways. Several human diseases have been attributed to such disturbances in balance, including cancer, diabetes, and inflammation. One of the targets of oxidant-mediated signaling is the mitogen-activated protein kinase (MAPK) cascade, which comprises highly conserved serine/threonine kinases and connects cell surface receptors with regulatory targets in response to various stimuli.14 MAPKs are also involved in mechanically induced signaling in various ocular cell types,15–17 including trabecular meshwork (TM), in which MAPK activation occurs in response to contraction and passive stretching.18 Members of the MAPK family may also have a critical role in TM cell apoptosis.19 Protein tyrosine kinase (PTK) was found to be involved in ciliary muscle contraction, and PTK inhibition caused TM relaxation and a decrease in IOP.20 We previously suggested that serine/threonine kinases take part in AH outflow control21 and showed that changes in MAPK protein expression and activation occur within the AH of a rat model of induced elevated IOP.22

It was suggested that there is a close and direct signaling effect between redox state and kinase/phosphatase activity.23 Dephosphorylation of specific phosphorylated sites can inhibit kinase activity. Three major families of phosphatases regulate phosphorylated proteins: protein-tyrosine phosphatase (PTP), serine/threonine phosphatase (PP), and dual-specificity phosphatase that cleaves both PTP and PP.24 A number of papers have provided insight into how PTPs might be transducers of oxidative stress conditions, leading to precise redox regulation of these enzymatic classes.25,26

Limited data are available regarding the presence of phosphatases in the ocular system; high levels of PP2A and PP1 were found in protein extracts of rabbit ciliary epithelium and the iris ciliary body, respectively, and in mouse retinas and lenses.27 Phosphatase expression is essential for optimal lens function,28 whereas the natural phosphatase inhibitor, okadaic acid, caused lens epithelial cell apoptosis by inhibiting PP1.29

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There are few data regarding the presence of phosphatases in the TM or AH. Measurable levels of PTP-zeta were found in a proteomics analysis of AH in cataract patients. Increased phosphatase activity at the TM or the ciliary muscles may contribute to a decrease in IOP and is a possible target for anti-glaucoma therapy.

We conducted this study to search for the presence and activity of phosphatases in the AH of patients with glaucoma and cataract to better understand the mechanisms affecting AH signaling in pathologic states. We further aimed to verify potential correlations between the redox state of the AH and the detected phosphatases.

Patients and Methods

Patients

The research followed the tenets of the Declaration of Helsinki, and the study protocol was approved before study initiation by the local ethics committees at Rebecca Ziv Hospital (Safed, Israel) and at Barzilai Medical Center (Ashkelon, Israel).

The study included 115 AH samples from patients diagnosed either with senile cataract alone (cataract group) or with senile cataract combined with pseudoexfoliation, primary open-angle glaucoma (POAG), or secondary open-angle glaucoma associated with exfoliation syndrome (glaucoma group). The cataract group consisted of 86 patients who were scheduled for cataract surgery and had senile cataract, normal IOP, and no evidence of glaucoma. The glaucoma group consisted of 29 patients with primary or secondary pseudoexfoliative open-angle glaucoma who were scheduled for cataract surgery with or without glaucoma surgery. All patients were Caucasians; the mean age was 74.4 ± 9.69 years for the glaucoma group and 72.78 ± 8.08 years for the cataract group. Patients with angle closure glaucoma or a history of surgery or ocular trauma were excluded. All patients underwent complete ophthalmologic evaluation that included medical history, best-corrected visual acuity, slit-lamp biomicroscopy with and without dilation, applanation tonometry, dilated funduscopy, and ophthalmoscopy of the optic disc. In addition, patients with glaucoma underwent gonioscopy and computerized visual fields. The glaucoma patients were treated with two or three IOP-reducing ophthalmic drops, including a beta blocker, an alpha agonist, a carbonic anhydrase inhibitor, and prostaglandin. Most of the glaucoma patients (26 of 29) had prostaglandin ophthalmic drops as part of their medical treatment.

Sample Collection

AH samples were collected at the Rebecca Ziv Hospital, Department of Ophthalmology, in Safed, Israel, and at the Barzilai Medical Center (Ashkelon, Israel). Similar preoperative local anesthesia techniques were used during surgery. Identical topical and systemic medications were used for all patients included in this study. AH was aspirated at the beginning of the surgical procedure through an opening of 1 mm at the limbus. A small amount of AH (15–80 μL) was withdrawn through the limbal paracentesis site using a 27-gauge needle on a tuberculin syringe, with special care to avoid blood contamination. The AH samples were transferred to premarked Eppendorf tube and kept frozen at −70°C.

Aqueous Humor Analysis

Protein Content. The total protein concentrations of the AH samples were determined according to the Bradford method, with bovine serum albumin as a standard.

Ferric Reducing Antioxidant Power Assay. Total antioxidant activity was measured by the ferric reducing antioxidant power (FRAP) procedure described by Benzie and Strain. Briefly, 180 μL freshly prepared FRAP reagent (37°C) was mixed with 18 μL distilled water and 6 μL tested sample. Readings at the absorption maximum (595 nm) were taken every 15 seconds up to 30 minutes using a microplate reader (model 680; Bio-Rad, Hercules, CA). Aqueous and methanolic solutions of known Fe(II) concentrations in the range of 0.1 to 2 mM (FeSO₄·7H₂O) were used for calibration.

Oxygen Radical Absorbance Capacity Assay. The oxygen radical absorbance capacity (ORAC) assay measures antioxidant scavenging function against the peroxyl radical induced by 2,2'-azobis-2-methylpropanimidamide dihydrochloride (AAPH) at 37°C, with fluorescein as a fluorescent probe. Loss of fluorescence was used to indicate the extent of damage caused by the reaction with the peroxyl radical. Fluorescein solution (170 μL, 60 nM final concentrations) and 3 μL of the sample were placed in a microplate well and incubated for 10 minutes (37°C). Then 20 μL AAPH (51.5 mM final concentration) was added, and fluorescence was recorded every minute in an infinite microplate reader (M200 Tecan Microplate; Tecan Group Ltd., Mannedorf, Switzerland) and appropriate software (Bio-Lynx Data Capture software; Bio-Lynx, Montreal, Quebec, Canada). Decay curves (fluorescence intensity versus time) were recorded, and the area between the two decay curves (with or without antioxidant) was calculated. The antioxidant trolox was used as a standard, and the final ORAC values were calculated using a regression equation between the known trolox concentration and the net area under the curve. ORAC values were expressed as trolox (μM) equivalents (TE) per sample (μL).

Ratio of Reduced Glutathione (GSH) to Oxidized Glutathione (GSSG) Content in the AH. The total cellular glutathione concentration was assayed by the GSSG-reductase and Ellman reagent (5,5-dithiobis(2-nitrobenzoic acid; DTNB) procedure. GSH was oxidized sequentially by DTNB and reduced by NADPH in the presence of glutathione reductase. Formation of the nitro-thiobenzoic acid was monitored by comparing the absorbance at 410 nm (microplate reader model 680; Bio-Rad) with that of standard samples of GSH in the same buffer.

Superoxide Dismutase Assay. The superoxide dismutase (SOD) activity of the AH was evaluated indirectly using the nitro blue tetrazolium assay. The reaction medium and the required amount of AH were illuminated for 10 minutes (25°C), and absorbance at 560 nm was subsequently measured (microplate reader model 680; Bio-Rad).

Analysis of the Presence of Cells within the AH. Fluorescence assay using laser excitation and emission wavelengths of 488 and 532 nm, respectively, was used to determine the cell content of AH samples with a microcapillary flow cytometer (Guava EasyCyte; Millipore, Billerica, MA) and analysis software (ViaCount; Millipore).

Assay of Serine/Threonine Phosphatase Presence and Activity. Serine/threonine phosphatase (PP2A) activity was assayed with a serine/threonine phosphatase assay kit (V2460; Promega, Madison, WI). Frozen AH samples were homogenized 1:10 (wt/vol) in ice-cold homogenization buffer with 1 mM phenylmethyl sulfonyl fluoride, 1 μM/ml leupeptin, and 1 μg/ml aprotinin. The AH homogenates were centrifuged at 20,000g for 20 minutes. Supernatants were desalted on small chromatography columns (Sephadex G-25; GE Healthcare, Piscataway, NJ). PP2C activity was measured against the same substrate peptide but in the presence of 11 μM okadaic acid and 10 mM MgCl₂. Reactions were initiated by the addition of AH, incubation for 10 minutes at 22°C, and termination by the addition of 50 μL malachite green dye reagent. The phosphatase activity was measured at 600 nm with the infinite microplate reader (M200; Tecan Group Ltd.) and the data capture software (Bio-Lynx). One unit of activity is defined as the amount of enzyme that catalyzes the release of 1 nmol phosphate per minute at 25°C ± 1°C. The serine/threonine phosphatase reaction was linear with respect to both time and protein concentration in the ranges used.

Assay of Tyrosine Phosphatase Activity. Phosphatase (PTPase) activity in the AH samples was determined using a commercial kit (V2471; Promega). Briefly, samples were incubated with 5 nmol each of two peptide substrates (ENDpYINASL and DADePpYIPQQG) in a total volume of 50 μL for 30 to 60 minutes in a 96-well plate. The PTPase reaction was stopped by the addition of 50 μL of a proprietary acid/molybdate-based dye reagent, and free phosphate was determined from the absorbance at 600 nm using an inorganic phosphate standard.
The PTPase reaction was linear with respect to both time and protein concentration in the ranges used.

**Enzyme-Linked Immunosorbent Assay.** Quantitative detection of alkaline phosphatase (ALP) was performed with an ALP ELISA kit (MaxiDiscovery; Bio Scientific, Austin, TX). Briefly, 100 μL AH sample (10 μg total protein), together with decreasing concentrations of ALP enzyme standard (600–75 U/mL) diluted in standard buffer, was added to microtiter plate wells precoated with an antibody to ALP. After incubation in 37°C for 90 minutes, the samples were removed and the wells were washed three times with wash solution and dried, and a second antibody directly conjugated to horseradish peroxidase was added. After incubation in 37°C for 60 minutes and wash, a signal was generated by the reaction of peroxidase with 3,5-tetramethylbenzidine for 15 minutes at room temperature. The developed color absorption was measured at 450 nm (microplate reader model 680; Bio-Rad). The intensity of the signal was proportional to the amount of PPs in the sample.

**Western Blot Analysis.** Equal amounts of protein samples (6 μg/well), determined according to the Bradford method, were heated at 95°C for 6 minutes and then loaded onto 1% sodium dodecyl sulfate–10% polyacrylamide gels. A prestained protein ladder (Bench Mark; Invitrogen Life Technologies, Carlsbad, CA) was used for molecular weight standards. After electrophoretic separation, the proteins were transferred to nitrocellulose membranes. Equal loading and appropriate transfer of each lane were confirmed by staining the membrane with Ponceau S solution (Sigma, St. Louis, MO). Immunodetection was performed with primary antibody against human pp2C (sc-1110; Santa Cruz Biotechnology, Santa Cruz, CA) 1:1000 and detected with a Western blot detection system (sc-2030; Santa Cruz Biotechnology, Santa Cruz, CA) 1:2000 overnight at 4°C, followed by a secondary antibody (Peroxidase-AffiniPure Bovine Anti-Goat IgG; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) 1:1000 and detected with a Western blot detection system (ECL Plus; Amersham Pharmacia Biotech, Buckinghamshire, UK), with exposure on X-ray film (Eastman Kodak, Rochester, NY). The films were analyzed by densitometry using an imaging system with corresponding software (Chemilmager 5500, Alpha Ease FC software; Alpha Innotech Corporation, San Leandro, CA).

**Statistical Analysis**

Data were analyzed with statistical analysis software (SPSS version 14.0; SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used for checking normality. ANOVA for independent samples was used when comparing results, and the χ² test was used for analysis of phosphatases that tested positive in the AH samples. Pearson’s correlation was used to check the correlation between variables. The statistical level of significance was *P < 0.05.*

### RESULTS

Cataract patients have been used in the present study as “controls” for comparison on the grounds that the samples are available, unlike “healthy” controls. The demographic and clinical features of the cataract and glaucoma groups are presented in Table 1. One hundred fifteen AH samples were obtained from patients 50 to 92 years of age who were enrolled in the study according to the inclusion/exclusion criteria as described in Methods. There were 29 primary open-angle glaucoma eyes (glaucoma group) and 86 senile cataract eyes (cataract group).

### Protein Content of the AH

The mean protein content of the AH samples was 2.07 ± 0.54 μg/μL. All tested groups had similar concentrations of proteins: 2.08 ± 0.72 μg/μL in the cataract group, 2.03 ± 0.32 μg/μL in the cataract + pseudoexfoliation (pxf) subgroup, 2.16 ± 0.52 μg/μL in the glaucoma group, and 2.00 ± 0.02 μg/μL in the glaucoma + pxf group.

### Oxidative State of the AH Samples

Oxidative damage is involved in the pathophysiology of both cataract and glaucoma. To look for correlations between the oxidative state of the AH samples, type of eye disease, and phosphatase levels, we evaluated the antioxidant levels, antioxidant enzyme concentration, and total antioxidant capacity of the samples. Antioxidant levels reflected by GSH levels and

### Table 1. Demographic and Clinical Features in the Cataract and Glaucoma Groups

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Women</th>
<th>Men</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract</td>
<td>72</td>
<td>44</td>
<td>28</td>
<td>72.49 ± 8.38</td>
<td>53</td>
<td>89</td>
</tr>
<tr>
<td>Cataract + pxf</td>
<td>14</td>
<td>5</td>
<td>9</td>
<td>74.21 ± 6.45</td>
<td>59</td>
<td>85</td>
</tr>
<tr>
<td>Cataract group</td>
<td>86</td>
<td>49</td>
<td>37</td>
<td>72.78 ± 8.08</td>
<td>53</td>
<td>89</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>18</td>
<td>7</td>
<td>11</td>
<td>71.67 ± 11.03</td>
<td>50</td>
<td>92</td>
</tr>
<tr>
<td>Glaucoma + pxf</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>78.91 ± 4.53</td>
<td>73</td>
<td>87</td>
</tr>
<tr>
<td>Glaucoma group</td>
<td>29</td>
<td>14</td>
<td>15</td>
<td>74.41 ± 9.69</td>
<td>30</td>
<td>92</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>65</td>
<td>52</td>
<td>73.21 ± 8.51</td>
<td>50</td>
<td>92</td>
</tr>
</tbody>
</table>

### Table 2. Summary of the Antioxidant Parameters of AH

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (μM)</th>
<th>GSSG (μM)</th>
<th>GSH/GSSG</th>
<th>SOD (μU/μL)</th>
<th>ORAC (μmol/μL Trolox equivalent)</th>
<th>FRAP (μmol/μL AA equivalent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract</td>
<td>0.203 ± 0.002*</td>
<td>0.242 ± 0.016</td>
<td>0.101 ± 0.015*</td>
<td>1.845 ± 0.097</td>
<td>40.16 ± 2.96</td>
<td>1049.26 ± 124.75</td>
</tr>
<tr>
<td>Cataract + pxf</td>
<td>0.252 ± 0.006*</td>
<td>0.237 ± 0.027</td>
<td>0.114 ± 0.034*</td>
<td>1.780 ± 0.262*</td>
<td>33.72 ± 3.84</td>
<td>723.05 ± 163.09</td>
</tr>
<tr>
<td>Cataract group</td>
<td>0.204 ± 0.002*</td>
<td>0.244 ± 0.014</td>
<td>0.103 ± 0.013*</td>
<td>1.839 ± 0.087</td>
<td>38.74 ± 2.47</td>
<td>994.89 ± 108.05</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>0.219 ± 0.006†</td>
<td>0.260 ± 0.039</td>
<td>0.061 ± 0.015†</td>
<td>1.876 ± 0.273†</td>
<td>35.93 ± 6.51</td>
<td>1389.74 ± 317.40</td>
</tr>
<tr>
<td>Glaucoma + pxf</td>
<td>0.016 ± 0.006†</td>
<td>0.243 ± 0.054</td>
<td>0.047 ± 0.014†</td>
<td>2.141 ± 0.306†</td>
<td>40.46 ± 6.04</td>
<td>1092.93 ± 183.85</td>
</tr>
<tr>
<td>Glaucoma group</td>
<td>0.018 ± 0.004†</td>
<td>0.252 ± 0.030</td>
<td>0.055 ± 0.010†</td>
<td>1.995 ± 0.200†</td>
<td>35.71 ± 4.81</td>
<td>1286.50 ± 215.54</td>
</tr>
</tbody>
</table>

Values are mean ± SD, and results were subjected to ANOVA.

*† Significant differences between the values (*P < 0.05).
the GSH/GSSG ratio were higher in the cataract group than in their matched glaucoma group (ANOVA, P < 0.05). Antioxidant enzyme concentration reflected by SOD was significantly higher only in the cataract + pxf versus the glaucoma + pxf (ANOVA, P < 0.05) group. Total antioxidant capacity as measured by ORAC and FRAP were similar through all the tested groups (Table 2).

**Evaluation of Phosphatase Activity in Human AH**

We evaluated the activity of three types of phosphatases, serine/threonine phosphatases (PP2A, PP2C) and tyrosine phosphatases (PTP). Only some of the tested samples in each group revealed active phosphatases; the percentage of positive phosphatase activity in each group is presented in Table 3. For all three phosphatases assayed, the proportion of positive results in the glaucoma group was significantly higher than that observed in the cataract group (PPase2A χ²(1) = 11.754, P < 0.01; PPase2C χ²(1) = 8.754, P < 0.01 **PTP χ²(1) = 11.073, P < 0.01**).

Phosphatase activity was found in all the tested groups, but there was no statistically significant difference among these groups. A trend of higher activity of the PPs in the cataract + pxf group was observed. In addition, all phosphatases showed lower levels of activity in the glaucoma group than in the cataract group (Fig. 1).

**Quantitative Detection of Phosphatases**

To directly measure the presence of PPs within the AH, ELISA and Western blot analysis were performed. General serine/threonine phosphatases were detected with an antibody directed against ALP. ALP levels were significantly higher in the glaucoma and in the glaucoma + PXF groups than in the cataract group, where it was undetectable (ANOVA, P < 0.01) (Fig. 2).

Semi-quantitative analysis of PPase2C in the AH from cataract and glaucoma patients revealed significantly higher concentrations of the enzyme in the AH from glaucoma patients (glaucoma 13,106 ± 1526 and cataract 7257 ± 645; Mann-Whitney U test, P = 0.012).

Semi-quantitative analysis of PTP revealed higher levels of the enzyme in the AH of glaucoma patients, though no statistically significant differences were observed between the two groups (glaucoma 4298 ± 1944 and cataract 2059 ± 1212; Mann-Whitney U test, P = 0.330) (Fig. 3).

**Statistical Correlations**

In the present study, we hypothesized that a relationship exists between the AH redox state and phosphatase levels and activity. Pearson correlation analysis revealed significant correlations between the two serine/threonine protein phosphatases 2A and 2C (r = 0.386; P < 0.01) and between the total protein tyrosine phosphatase and PPase2A (r = 0.204; P < 0.05). Within the redox state analysis, we found a significant correlation between total antioxidant activity (FRAP) and PTP levels (r = 0.244; P < 0.049; Table 4).

**DISCUSSION**

IOP is the major risk factor of POAG; it is well recognized that a controlled balance between AH production and drainage is
critical for normal IOP maintenance. Therefore, the major efforts in the treatment of POAG are concentrated on modulating AH production and drainage, either with medications or surgery. The focus on IOP as the only risk factor has, however, left several questions unanswered: Why does the reduction in IOP not stop the progression of glaucomatous optic neuropathy in all patients? Why is it necessary to lower the IOP to extremely low values in some patients to stop the progression of the disease? What is the cause of normal tension glaucoma? New approaches for treating glaucoma are therefore needed to address these and other open questions. Although the exact pathogenesis of glaucoma remains unclear, it is likely that alteration in AH protein composition triggers signaling molecules that could modify the TM, increasing resistance to outflow and leading to POAG.36

In the present study, we have shown that phosphatases from the serine/threonine family and PTP are present and active in the AH of cataract and POAG patients. A significant association was found between PPase2A and PPase2C and between PPase2A and PTP. This is the first time, to our knowledge, that a direct analysis of phosphatases within the AH system, using specific antibodies, was performed, adding to the single report about the presence of PTP-zeta, which was found through proteomic analysis in human AH.30

In our previous studies we detected MAPK proteins in rat AH and showed that their expression levels were affected by the degree of IOP.22 Although the sources for MAPK remained obscure, we speculated that phosphatases—the counterbalancing enzymes of these kinases—might be present in AH as well. Results of the present study confirmed this hypothesis and may contribute to the hypothesis of Coca-Prados and Escribano37 that paracrine and autocrine effects within the well. Results of the present study confirmed this hypothesis, suggesting that phosphatases and MAPK might be present in AH as well. These results suggest that the pathologic condition in cataract and POAG might cause an elevation in phosphatase levels in human AH.

Although there are some significant differences between the AH content of cataract patients and the AH content of POAG patients, and although exfoliation syndrome contributes, in some cases, to the differences observed in these conditions,40–43 cataract and POAG both share oxidative stress components in their pathophysiology that might mask some differences between the two groups. Moreover, most POAG patients were treated with local prostaglandin ophthalmic drops (latanoprost or Travoprost), which involve reactive oxygen species production in their mechanism of action and might affect the total antioxidant status. In the present study, FRAP and ORAC assays performed on the cataract and glaucoma groups did not reveal significant differences in oxidative stress markers or in redox state analysis. The small group numbers, the backgrounds of the patients, and treatment with prostaglandin eyedrops may be the reason for our results. Statistically significant differences in glutathione levels and in the GSH/GSSG ratio between POAG and cataract patients was similar to those of previous studies.44 A significant correlation was found between the redox state of the AH as expressed by the FRAP method and PTP levels, that was undependable on the patients’ clinical background and despite prostaglandin treatment. This correlation matches the known effects of the redox states on phosphatase and kinase regulation.45,46

The MAPKs play key roles in the regulation of endothelial cell function through the phosphorylation of a number of cytosol and nuclear targets.77 Similarly, in our study, we can assume that overexpression of MAPK phosphatases enhances adhesion molecule expression and might be an apoptosis protecting mechanism.88 Changes in the phosphatases and MAPK activity in the AH could occur as a consequence of an altered functioning of the TM endothelium. Understanding the role of the phosphatase activity in the AH will contribute to our knowledge of the pathogenesis of POAG and may provide us with promising approaches for the discovery of new targets for therapeutic intervention and for the development of novel, more efficacious, treatment modalities for POAG.

**References**


